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INHIBITORS OF GERMINATION AND GROWTH FROM RED ELDERBERRY: A LABORATORY EVALUATION

by

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ABSTRACT

Field observations on subalpine rangeland had indicated that emergence and growth of planted grasses was reduced in the vicinity of red elderberry (*Sambucus racemosa* var. *microbotrys*). To determine whether antibiotic substances were involved in this effect, aqueous extracts of various parts of elderberry were tested for inhibition of germination and seedling growth of grasses and other associated species. Inhibition of germination was weak, and was diminished further or eliminated by such treatments of the extract as moderate dilution or adsorption of the active component(s) on charcoal. In pot culture in the greenhouse, seedling growth of grasses was not reduced by a surface mulch of elderberry leaves. It was concluded that the observed depression of emergence and growth that was observed in the field could not be attributed to the liberation of toxic substances from elderberry, unless special conditions exist in the field which are not duplicated in the laboratory or greenhouse.

EFFECTS AND IMPORTANCE OF INHIBITORS

One plant may inhibit the growth of other plants in its vicinity by liberating inhibitory substances into the environment (Borner 1960). This property may help determine the importance of individual species in a community: the inhibitor-producing plants will

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face reduced competition, and they will grow and spread at the expense of other species sensitive to the inhibitory substances.

Inhibitory effects of aqueous leachates from crop plants have been studied extensively, but only recently has research been directed toward the possible role of inhibitors in determining species composition and importance in the vegetation of rangelands. Muller et al. (1964) studied the effect of volatile growth inhibitors in aromatic shrubs in California. Reid et al. (1963) and Wilkie and Reid (1964) demonstrated the inhibitory effect of foliar leachates from four species of sagebrush on germination and growth of several crop species and native grasses. Jameson (1966) found that juniper litter inhibited the growth of blue grama.

A study of the possible production of growth inhibiting substances in red elderberry (Sambucus racemosa var. microbotrys (Hitchcock et al. 1959)) was prompted by field observations which suggested that germination of several species of introduced grasses had been delayed or prevented in plots where elderberry had been growing. In these plots, few grass seedlings emerged within areas from which elderberry had been removed. It therefore seemed desirable to determine the effectiveness of aqueous extracts of various parts of elderberry for inhibitory influence on germination and early seedling growth of several native and introduced species.

Red elderberry (figure 1), also called bunchberry elder, is one of the most common of the western elderberries in the central Rocky Mountains and the general Intermountain region. It extends from southwestern Montana eastward to South Dakota, southward to New Mexico, and westward to Arizona and Nevada, chiefly on moist sites in the upper ponderosa pine, aspen, spruce, and subalpine belts (U.S. Dep. Agr. 1937).

METHODS AND MATERIALS

Collections of aerial shoots and rhizomes of red elderberry were made at approximately 8,000 feet near Strawberry Reservoir on the Uinta National Forest, and in Logan Canyon on the Cache National Forest, Utah. Fresh plant material was processed the day of collection. Otherwise, the plant parts were dried in a current of air at room temperatures of 22-25°[2] for 72 hours.

Preliminary Experiments

In preliminary experiments, several methods of extraction were tested. Air-dry elderberry leaves were homogenized in distilled water for 1 minute, and the homogenate was filtered and refrigerated until used in experiments on the same day. In other preliminary experiments, fresh leaves and ground portions of air-dry stems and rhizomes were extracted. Blending time and length of time that the extract was in contact with the leaf tissue were also tested as factors affecting the inhibitory activity of the extract. All methods of extraction produced essentially the same results.

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[2] All temperatures shown are °C.

Figure 1.--A partially excavated clone of red elderberry from the Uinta National Forest. Aerial stems are in the deciduous condition.

Extracts made from air-dry leaves were as potent as those from fresh leaves and surpassed those from air-dry stems and rhizomes. The extract used for the results reported in this paper was prepared from air-dry leaves that were homogenized for 1 minute before filtration.

Seeds of grasses used in studies of germination and seedling growth were obtained from a commercial source, and included species commonly used in the revegetation of subalpine rangeland. The species used in preliminary experiments were intermediate wheatgrass (Agropyron intermedium), tall oatgrass (Arrhenatherum elatius), smooth brome (Bromus inermis), orchard grass (Dactylis glomerata), and timothy (Phleum pratense). Seeds of native forbs were collected from aspen or grass-forb communities on the Uinta National Forest during the summer of 1965. These species were chosen because they grow in the same areas as red elderberry. All seeds were stored in vapor-tight bottles at 2° until used, except for the seeds of Polemonium foliosissimum, which were stored under room conditions.

In preliminary experiments, the effect of a leaf extract (10 g./100 ml.) on the germination of the five species of grass was determined according to the method of Lawrence and Kilcher (1962). Germination of all species was inhibited to a highly significant extent (0.1-percent probability level). However, since there was doubt that such a concentration could be attained in the field, and since there may be osmotic effects at this concentration (Anderson and Luocks 1966), the air-dry weight of leaves used in preparation of extracts was reduced by two-thirds (to 3.3 g./100 ml.) for the experiments reported below.

Later Tests

Additional germinative tests were run on six grasses and four forbs (tables 1 and 2). Seeds (approximately 50 per dish) were placed in 9-cm. petri dishes on double layers of Whatman No. 1 filter paper[3] wetted with 4 ml. of distilled water or extract. The dishes with seeds were placed under 8-hour photoperiods at 22° and 16-hour dark periods at 17°. Temperatures were controlled to within 1°. Seeds were inspected daily for germination (growth of the embryonic root through the covering layers), and final germination percentages were determined when no additional germination had occurred for 3 days. Dishes and seeds under each treatment were replicated, so that results given in the text and tables of this paper represent the means of four determinations plus or minus standard deviations. The data on percentages of seed germination were analyzed statistically, with and without arcsine transformations. Indications of significance were the same by either method.

Experiments testing seedling growth of three grasses were done under greenhouse conditions in 6-inch clay pots filled with a sandy loam. For each species, four pots each were used for treatment and control. Following the planting of seeds at a depth of one-half inch (12 per pot, thinned to 4), 10 grams air-dry weight of elderberry leaves were crushed and distributed uniformly over the surface of each treated pot. The soil surface of control pots was left bare, since any other kind of mulch might have had unknown promotive or inhibitory effects. The soil surface was watered on alternate days with 250 ml. of tapwater.

RESULTS

Seed Germination

In tests of germination of six species of grass (table 1), germination rates and germination percentages were reduced for all species, except pubescent wheatgrass (rate) and smooth brome (final percentage). In further tests with germination of foxtail (the most sensitive species), pretreatment of the extract by storage for 6 days at 2° or by boiling for 1 minute had no effect on the inhibition of germination. Inhibition was eliminated when the extract was clarified by filtering it through activated charcoal; final percentages of germination were 96% ± 1 for treated samples, and 94% ± 1 for controls. Similarly, a 1:1 dilution of the untreated extract completely eliminated the inhibition (95% ± 2).

The extract and dilutions generally inhibited germination in native forbs (table 2). A comparison of mean percentages of germination of seeds imbibed in distilled water or various dilutions of the extract showed that, except for the zero and 1:1 dilutions for *Aquilegia*, all differed significantly from all other treatments for each species at the 5-percent level.

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[3]Use of trade names herein is solely for identification and does not imply endorsement by the U.S. Forest Service.

Table 1.--Final percentages of germination of grass seeds imbibed on filter paper in distilled water (control) and in extract of elderberry leaves

Species	Percent germination: Water	Percent germination: Extract
Intermediate wheatgrass Amur (*Agropyron intermedium*)	[1] 100 (2)	87 ± 2 (3)**
Meadow foxtail (*Alopecurus pratensis*)	97 ± 1 (5)	64 ± 4 (7)**
Pubescent wheatgrass Topar (*Agropyron trichophorum*)	96 ± 1 (4)	84 ± 3 (4)**
Slender wheatgrass (*Agropyron trachycaulum*)	100 (3)	96 ± 1 (5)**
Smooth brome Manchar (*Bromus inermis*)	84 ± 2 (3)	87 ± 2 (4)
Tall oatgrass Tualatin (*Arrhenatherum elatius*)	76 ± 4 (3)	69 ± 3 (4)*

[1] Numbers in parentheses indicate days to one-half of the final germination percentage.

*Difference between control and seeds treated with extracts is significant at 5-percent level of probability.

**Difference significant at 1-percent level of probability.

Table 2.--Germination percentages of seeds of native forbs imbibed in water or extract of elderberry leaves

Species	Extract	Germination
Colorado columbine (*Aquilegia coerulea*)	H_2O	60 ± 4
	1:4	54 ± 4
	1:1	28 ± 2
	0	31 ± 2
Fendler meadowrye (*Thalictrum fendleri*)	H_2O	74 ± 2
	1:1	83 ± 1
	0	61 ± 3
Leafy polemonium (*Polemonium foliosissimum*)	H_2O	27 ± 3
	1:1	22 ± 2
	0	8 ± 1
Nettleleaf horsemint (*Agastache urticifolia*)	H_2O	56 ± 2
	1:4	48 ± 4
	1:1	34 ± 3
	0	21 ± 1

Seedling Growth

The mulch of elderberry leaves had no detrimental effect on growth of seedlings of foxtail, intermediate wheatgrass, and slender wheatgrass during 1 month; the fresh and dry weights of treated plants equaled or exceeded those of the controls. Any inhibitory effect of the mulch was probably balanced or outweighed by its beneficial effects under greenhouse conditions.

DISCUSSION

To be effective in nature, an inhibitory substance must be strong enough to produce an effect despite a large dilution in the environment, and despite other losses due to adsorption, microbial action, and chemical reaction with other substances. Preliminary field observations suggested that red elderberry somehow inhibited the emergence of introduced grasses, at least during the first year after its removal. However, the inhibitory effect produced in laboratory tests lacked potency; it was diminished by moderate dilution; it was eliminated by filtration through charcoal; and it was not effective in pot culture under greenhouse conditions. Thus, its effectiveness under field conditions appears doubtful unless special conditions exist in the field that were not duplicated in the laboratory.

Elderberry is a rhizomatous shrub (figure 1), and a particular plant may occupy the same site for many years. One rhizome examined had at least 58 annual rings and probably was much older. Therefore, it is possible that inhibitory substances leached from the leaves and rhizomes might be concentrated in the soil in the immediate vicinity of the group of aerial stems. Perennial species are seldom found within the area occupied by well-defined clumps of aerial stems of red elderberry. However, factors of competition other than production of inhibitory substances may be acting to exclude other species.

Laboratory and greenhouse tests did not indicate that inhibitory substances in elderberry are sufficiently potent to be effective under field conditions. To properly determine the existence and ecological significance of any inhibitor, extensive field studies would be needed to establish the presence of the inhibitor in the soil directly beneath and at various distances from clones of elderberry. Association analysis would also be required to determine the spatial relations between red elderberry and associated species.

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